

## Comparison of Broth Microdilution Method Using *Haemophilus* Test Medium and Agar Dilution Method for Susceptibility Testing of *Eikenella corrodens*

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**Susceptibility testing of *Eikenella corrodens* is usually performed by a Mueller-Hinton sheep blood agar dilution (AD) method. However, this method is impractical for testing only a few strains. We compared AD with the broth microdilution method using *Haemophilus* test medium (HTM) in order to determine the susceptibility of 36 clinical isolates of *E. corrodens* to eight antimicrobial agents. MICs obtained by the HTM method yielded 95.5 and 84% agreement (within 2 and 1 log<sub>2</sub> dilutions, respectively) with those obtained by AD. The HTM method with incubation in CO<sub>2</sub> for 48 h was highly reproducible and constitutes an easy alternative for antimicrobial susceptibility testing of *E. corrodens*.**

*Eikenella corrodens* is a fastidious, slow-growing, gram-negative rod that has been increasingly recognized as a pathogen in a wide variety of infections (2, 12). Increased isolation of  $\beta$ -lactamase-producing strains and frequent resistance to many antimicrobial agents make necessary the routine determination of the susceptibility of this pathogenic bacterium to different antimicrobial agents (2, 3, 5, 6, 9, 10, 13, 14). Susceptibility testing has usually been performed by a sheep-blood-based agar dilution (AD) method which is tedious, time-consuming, and impractical for laboratories testing only a few strains (3). In this study we compare the AD method with a broth microdilution method using *Haemophilus* test medium (HTM) to determine the susceptibility of *E. corrodens* to eight frequently used antimicrobial agents.

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Thirty-six nonduplicate, recent clinical isolates of *E. corrodens* were tested. All strains were identified by standard microbiological methods (2, 7). The sources of the isolates were as follows: abscesses ( $n = 13$ ); wounds ( $n = 9$ ); blood ( $n = 4$ ); peritoneal fluid ( $n = 4$ ); tissue biopsy ( $n = 3$ ); and pleural fluid, umbilical tissue, and whitlow ( $n = 1$  each).  $\beta$ -Lactamase-producing strains were detected by the chromogenic cephalosporin test (nitrocefin) (Cefinase; BBL Microbiology Systems, Cockeysville, Md.). The isolates were maintained in 10% skim milk at  $-70^{\circ}\text{C}$  until ready for use and then subcultured three times on 5% sheep blood Columbia agar plates for 48 h at  $35^{\circ}\text{C}$  in CO<sub>2</sub> before susceptibility studies were performed. These cultures were used for inoculum preparation. The following antimicrobial agents were used as powders of known potency: ampicillin, cephalothin, cefotaxime, and tetracycline, obtained from Sigma Chemical Co., St. Louis, Mo.; amoxicillin and clavulanic acid, obtained from SmithKline Beecham, Worthing, United Kingdom; cefoxitin and imipenem, obtained from Merck, Sharp and Dohme, Madrid, Spain; and ciprofloxacin,

obtained from Bayer AG, Barcelona, Spain. All antimicrobial solutions were freshly prepared before use. AD MICs were determined by the standard procedure (3, 11) with Mueller-Hinton agar plates supplemented with 5% sheep blood incorporating twofold increments of the various antimicrobial agents, with concentrations varying from 128 to 0.001  $\mu\text{g/ml}$  depending on the antimicrobial agent being tested. Inocula consisting of  $10^4$  CFU/ml/spot were applied to the surfaces of the plates with a Steers replicator. Control plates without antimicrobial agents were also inoculated before and after each series of antibiotic-containing plates. All plates were incubated at  $35^{\circ}\text{C}$  in 5% CO<sub>2</sub> for 48 h and then examined. *Staphylococcus aureus* ATCC 29213 and *Enterococcus faecalis* 29212 were used as control strains.

MICs were also determined by the broth microdilution method according to National Committee for Clinical Laboratory Standards guidelines (11) with HTM broth made in-house. Plastic microdilution trays contained the antimicrobial agents in serial twofold dilutions from 128 to 0.001  $\mu\text{g/ml}$ , depending on the antimicrobial agent being tested. Inocula were prepared in HTM broth from cultures grown on Columbia agar with 5% sheep blood. The final concentration was  $5 \times 10^5$  CFU/ml. All microdilution trays were prepared in duplicate and incubated at  $35^{\circ}\text{C}$ ; one series was incubated in air, and the other was incubated in 5% CO<sub>2</sub>. Readings for both series were performed at 24 and 48 h. In order to assess reproducibility, 16 strains were tested in triplicate. Reproducibility was calculated as the percentage of MICs within a range of 3 log<sub>2</sub> dilutions. *Haemophilus influenzae* ATCC 49247 was used as a control strain.

The antimicrobial susceptibilities of *E. corrodens* as determined by AD and by broth microdilution under two different incubation conditions (air and CO<sub>2</sub>) and with two different reading times (24 and 48 h) are shown in Table 1. HTM broth provided good support for the growth of all *E. corrodens* isolates, especially when the microtrays were incubated in CO<sub>2</sub> for 48 h (C48-HTM). Only two strains failed to grow in air after 24 h. Results obtained by both methods show that all strains were uniformly susceptible to amoxicillin-clavulanate, cephalothin, cefoxitin, cefotaxime, imipenem, tetracycline, and ciprofloxacin according to National Committee for Clinical Labo-

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TABLE 1. Comparative susceptibilities of 36 strains of *E. corrodens* to antimicrobial agents by the AD and HTM broth microdilution methods

Antimicrobial agent	AD		HTM broth microdilution							
	Range <sup>d</sup>	MIC <sub>90</sub> <sup>a</sup>	24 h, O <sub>2</sub> <sup>b</sup>		48 h, O <sub>2</sub>		24 h, CO <sub>2</sub>		C48-HTM	
			MIC <sub>90</sub>	≤2 dil <sup>c</sup>	MIC <sub>90</sub>	≤2 dil	MIC <sub>90</sub>	≤2 dil	MIC <sub>90</sub>	≤2 dil
Ampicillin	0.25–64	2	1	53	1	75	1	94	1	97
Amoxicillin-clavulanate	0.12–1	0.5	0.25	92	0.25	97	0.5	100	0.5	100
Cephalothin	4–16	16	8	94	8	100	8	100	16	100
Cefoxitin	0.25–4	2	0.25	59	0.5	64	0.5	69	1	83
Cefotaxime	0.006–0.06	0.03	0.006	65	0.01	100	0.01	92	0.01	100
Imipenem	0.12–0.5	0.5	0.25	47	0.5	69	0.5	67	0.5	78
Tetracycline	0.25–2	1	1	91	2	86	0.5	92	2	97
Ciprofloxacin	0.006–0.01	0.01	0.006	94	0.01	97	0.01	97	0.01	97
Overall				74		86		89		94

<sup>a</sup> MIC<sub>90</sub>, the MIC at which 90% of the isolates are inhibited, expressed in micrograms per milliliter.

<sup>b</sup> Two strains failed to grow in air after 24 h.

<sup>c</sup> ≤2 dil, percentages of correlation of MICs obtained by the AD and HTM broth microdilution methods within 2 dilutions of each other.

<sup>d</sup> Expressed in micrograms per milliliter.

ratory Standards criteria (11); however, cephalothin was the least-active compound, with more than 50% of strains requiring 8 µg/ml for inhibition. There were two β-lactamase-producing strains (5%); the MICs of ampicillin for these isolates were 16 and 64 µg/ml. AD and HTM broth microdilution MICs were generally similar for all agents tested, especially with C48-HTM. Overall, MICs obtained by C48-HTM yielded 95.5% agreement (within 2 log<sub>2</sub> dilutions) with those obtained by AD. Agreement was 84% within 1 log<sub>2</sub> dilution (Table 2). Analysis of percentage of agreement for individual antimicrobial agents indicated no significant tendencies for amoxicillin-clavulanate and cephalothin; however, ampicillin, cefoxitin, and cefotaxime MICs tended to be 1 log<sub>2</sub> dilution lower with C48-HTM than with AD; conversely, tetracycline and ciprofloxacin MICs tended to be 1 log<sub>2</sub> dilution higher. Imipenem MICs varied widely, ranging from –4 to +3 dilutions, although 77.8% of the strains were within 2 log<sub>2</sub> dilutions. Reproducibility was 93%, regardless of incubation conditions and the antimicrobial agent used.

The AD technique is currently the method of choice for susceptibility testing of *E. corrodens*; however, it is tedious and time-consuming and impractical for laboratories testing only a few strains. The broth microdilution method has become increasingly popular, and many laboratories test susceptibility of most microorganisms by this technique (1). In 1983, Goldstein et al. (4) described a broth microdilution technique with a

filtrate of laked sheep blood which compared favorably with the AD method. However, the procedure was tedious, since it required lysed sheep blood. HTM was developed in 1987 in order to satisfy the complex growth requirements of *Haemophilus* spp. (8). This medium also allows the growth of many fastidious microorganisms; however, it has never been used for antimicrobial susceptibility testing of *E. corrodens*. In our study, the HTM broth microdilution method provided good support for growth of *E. corrodens* strains and was easily interpretable and highly reproducible. The MICs showed a high correlation with those obtained by the AD technique, especially with C48-HTM. However, the HTM broth microdilution method has not proved to be reliable for testing susceptibility to imipenem and cefoxitin. Although the HTM broth used in the present study was prepared in-house, this medium can be purchased commercially. On the basis of our results and the commercial availability of the HTM broth, we recommend the HTM broth microdilution method for antimicrobial susceptibility testing of *E. corrodens*.

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TABLE 2. Distribution in differences of MICs of antimicrobial agents determined by AD and HTM broth microdilution with C48-HTM

Antimicrobial agent	% of isolates for which MICs differ by indicated dilutions(s)							% within 1 log <sub>2</sub> dilution
	≤–3	–2	–1	0	+1	+2	≥+3	
Ampicillin	0	8.3	47.2	36.1	8.3	0	0	91.6
Amoxicillin-clavulanate	0	2.8	11.1	55.6	27.8	2.8	0	94.5
Cephalothin	0	2.8	25	52.8	19.4	0	0	97.2
Cefoxitin	13.9	8.3	30.6	36.1	8.3	2.8	0	75
Cefotaxime	0	13.9	47.2	36.1	2.8	0	0	86.1
Imipenem	19.4	11.1	25	11.1	8.3	19.4	2.8	44.4
Tetracycline	0	0	19.4	47.2	19.4	13.9	0	86.1
Ciprofloxacin	0	0	16.7	44.4	36.1	2.8	0	97.2

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